Supporting Information

SI Materiels and Methods

Endocytosis and recycling assays. To assay endocytosis, OP9 cells stably expressing one of the three Dll1 derivates were labelled on ice for 40 min with the reversible reagent NHS-SS-biotin (0.5 mg/mL in PBS). After a 20 min incubation at 37°C, biotin was stripped by three 25 min incubations with 50 mM MesNa (Sodium 2-mercapto-ethanesulfonate) in TNEB buffer (20 mM Tris pH 8.3; 150 mM NaCl; 1 mM EDTA; 0.2% BSA) on ice. Cells were then lysed in RIPA buffer (50 mM Tris pH 7.5, 150 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.5% DOC), and biotinylated species were purified onto streptavidin-agarose and analyzed by immunoblotting with anti-Dll1CT or anti-Dll3ic antibody. To assay recycling, OP9 cells stably expressing a Dll1 derivate were labelled on ice with NHS-SS-biotin, incubated for 20 min at 37°C and biotin was stripped with MesNa as described above. Cells were incubated for 10 min on ice with 5 mg/mL iodoacetamide in TNEB. After a second incubation at 37°C for 10, 20 or 30 min, cells were treated or not with MesNa, lysed in RIPA buffer, and biotinylated species were purified onto streptavidin-agarose and analyzed by immunoblotting with anti-Dll1CT or anti-Dll3ic antibody. Immunoreactivity was quantified using Quantity One software (Bio-Rad Laboratories). For each time point, the amount of recycled ligand was estimated by substracting the amount measured in MesNa treated cells from that in non treated cells and expressed as a percentage of endocytosed ligand.

SI figure legends

Fig. S1. Measurement of half-life of surface biotinylated Dll1 and Dll1K17R. OP9 cells expressing Dll1 or Dll1K17R were biotinylated at 4°C and shifted to 37°C during 1, 2, 3, 4, or 5 hours as indicated. Whole cell extracts were incubated with streptavidin-agarose and biotinylated species were analyzed by immunoblotting with anti-Dll1CT antibody. Quantitation was performed after scanning of the film using Quantity One program. The percentage of biotinylated ligands at a given time point vs that at time 0 is shown.

Fig. S2. Notch1 is taken up by Dll1-expressing cells. OP9 cells expressing VSV-tagged Dll1 were co-cultured with MEFs expressing HA-tagged Notch1 for 24 hours. Both tags are in the extracellular domain of the molecules. After fixation, cells were permeabilized with

detergent (a-c) or not (d-f). Dll1 was detected using a Cy3-coupled anti VSV antibody (red) and Notch1 using an Alexa488-coupled anti HA antibody (green). Hoechst staining is shown. Overlay: superposition of the two stainings.

Fig. S3. Notch1 is found associated with endocytic vesicles into Dll1-expressing cells. OP9 cells expressing VSV-tagged Dll1 were co-cultured with MEFs expressing HA-tagged Notch1 for 24 hours. Cells were incubated for 4 hours at 37°C in the presence of 4 mg/mL of Cascade Blue dextran (Molecular Probes) (blue in overlay panel). Then cells were then washed, fixed and permeabilized. Dll1 (red) was visualized with a rabbit anti Dll1CT and an Alexa647-coupled secondary antibody (Molecular Probes). Notch (green) was detected with a mouse anti HA antibody (HA-11, Covance) and a Cy3-coupled secondary antibody (Sigma). Overlay: superposition of the three stainings. Insert represents an enlarge view (12 fold) of the boxed region. Scale bar: 20 μm.